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DEFENSIVE BEHAVIOR AGAINST TERTIARY PARASITISM BY THE LARVA OF *DENDROCERUS CARPENTERI*[1] AN APHID HYPERPARASITOID[2]

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Abstract.—Bennett, Alfred W. and Daniel J. Sullivan, S.J., Department of Biological Sciences, Fordham University, Bronx, New York 10458.—Using the pea aphid as the host and *Aphidius smithi* as the primary parasitoid, the ovipositional behavior of the aphid hyperparasitoid, *Dendrocerus carpenteri*, is described. Laboratory experiments indicate that the possibility of tertiary parasitism by a 2nd *Dendrocerus* is greatly reduced to an average of 6.0% during the last 9 days of the development of the 1st *Dendrocerus* within the mummy. It is suggested that *Dendrocerus* has evolved a defensive behavior which deters tertiary parasitism at least by its own species. The 4th instar larva and the prepupa have a conical process at the posterior end which enables it to twitch violently and move about in the mummy when probed by the ovipositor of a 2nd *Dendrocerus*, thus inhibiting oviposition.

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Introduction

Members of several families of Hymenoptera are aphid hyperparasitoids or secondary parasitoids in that they attack, not the aphids themselves, but the primary parasitoid which is developing inside either a live aphid or the "mummy" (a dead aphid). This secondary parasitoid can in turn be attacked by another hyperparasitoid which would result in tertiary parasitism. This was demonstrated by Sullivan (1972) with the aphid hyperparasitoid, *Asaphes californicus* Girault, which successfully attacked and oviposited on another aphid hyperparasitoid, *Alloxysta victrix* (Westwood). The progeny of the *Asaphes* fed on the *Alloxysta* larva inside the mummy and eventually emerged as an adult 21 days later. He noted, however, that such tertiary parasitism diminished with time as the *Alloxysta* developed to the pupal stage. These were but two of a complex of five different families of hyperparasitoids which were studied in the field (Sullivan and van den Bosch 1971). Another species of hyperparasitoid, *Asaphes lucens* (Provancher), will even attack a developing larva of its own species. Thus this type of tertiary parasitism can be both interspecific and intraspecific. In the laboratory experiments described herein, one such aphid hyperparasitoid, *Dendrocerus carpenteri* (Curtis), exhibits a defensive behavior as a 4th instar larva and prepupa. This behavior reduces the possibility of it being parasitized and thus avoids tertiary parasitism.

Materials and Methods

Apterous, viviparous pea aphids, *Acyrthosiphon pisum* (Harris), were reared in the laboratory on broad bean plants, *Vicia fava* L. (Windsor variety), in a Percival bioclimatic chamber (E-54U). The daytime regime had a photoperiod of 16 hr, a temperature of 21.1 ± 0.6°C, and a relative humidity of 75 ± 5%. At night, the photoperiod was 8 hr, with a temperature of 15.5 ± 0.6°C and 85 ± 5% RH.

The primary parasitoid was *Aphidius smithi* Sharma and Subba Rao. After mating, 2–4 females were introduced into a glass "stinging-tube" containing a cut broad bean stem and 10–15 pea aphids. The stinging tube was kept in the bioclimatic chamber for 6 hr. At the end of that time, the wasps were removed, and the aphids placed on growing bean sprouts and returned to the bioclimatic chamber.

After 8 days, mummies were formed, and on the 9th day they were removed from the plant by cutting the leaf in a circle around the mummy in order to avoid damaging the mummy itself and to allow sufficient leaf substrate on which the *Dendrocerus* female could stand. Each mummy was then placed separately in a plain, uncoated Dixie Cup container (No. 2168-SE) and sealed with a clear plastic cover (Dixie No. 3068). The hyperparasitoid which was used, had formerly been called *Lygocerus niger* (Howard, 1890), but Dessart (1972) compared the Fordham University specimens with those in Europe and the U.S. National Museum of Natural History (Systematic Entomology Laboratory). He determined that they are the same species, viz.—*Dendrocerus carpenteri* (Curtis, 1829).

One mated *Dendrocerus* female was then introduced into the container, and her oviposition behavior observed. When egg deposition onto the *Aphidius* larva was completed, this 1st *Dendrocerus* was removed. Unsuccessful hyperparasitization could easily be determined by the fact that the *Aphidius* would continue its development and eventually emerge as an adult. In such cases, these replicates were discarded.

In the laboratory experiments on tertiary parasitism, although the developing *Dendrocerus* No. 1 could be attacked over a period of 15 days while developing inside the mummy (cf. Life Cycle—*Table 1*), Days 0–1–2–3 were omitted from the experiments because of the difficulty in determining whether the adult which eventually emerged was the progeny of the 1st or 2nd *Dendrocerus* female. Hence, only 12 test-days were used (Days 4–15), when the emergence of *Dendrocerus* No. 1 or 2 could be accurately determined. Normally the adults emerge on the 16th day after oviposition.

On each of the 12 test-days, the same procedure was followed, viz.—a 2nd mated *Dendrocerus* female was introduced into the container having a

Table 1. Composite life cycles of the primary parasitoid, *Aphidius smithi*, and the hyperparasitoid, *Dendrocerus carpenteri*, under experimental laboratory conditions.

Day	*Aphidius smithi*		
0	Egg deposited in aphid		
1			
2	1st larval instar		
3			
4	2nd larval instar		
5			
6	3rd larval instar		
7			
8	Host aphid mummified	Day	*Dendrocerus carpenteri*
9		0	Egg deposited on *Aphidius*
10	Prepupa (meconium voided)	1	1st larval instar
11	Pupa	2	
12	Adult emerges[a]	3	2nd larval instar
		4	
		5	3rd larval instar
		6	
		7	4th larval instar
		8	
		9	Prepupa (meconium voided)
		10	Pupa
		11	
		12	
		13	
		14	
		15	
		16	Adult emerges[b]

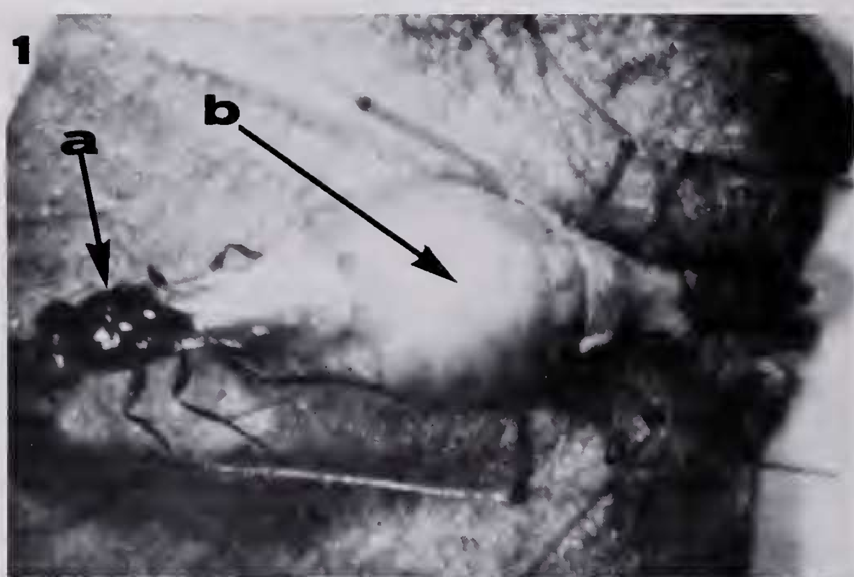
[a] In these experiments, because of hyperparasitization by *Dendrocerus*, the *Aphidius* is attacked on Day 9, and does not develop to an adult.

[b] Similarly, if *Dendrocerus* No. 1 is successfully hyperparasitized by *Dendrocerus* No. 2 during the experimental test-days (4–15), it will not develop to an adult.

mummy which had been previously hyperparasitized by the 1st *Dendrocerus*. The 2nd *Dendrocerus* female was removed after normal drilling and possible oviposition had been completed. Each mummy was then left undisturbed in its container, and returned to the bioclimatic chamber. At least 100 replicates were done for each of the 12 test-days (4–15).

Results and Discussion

The ovipositional behavior of *Dendrocerus carpenteri* has the following sequence. The female approaches the mummy and antennates it in a rather aggressive manner. She then walks on top of the mummy, but almost immediately walks off it at the spot where she will eventually drill a hole. This



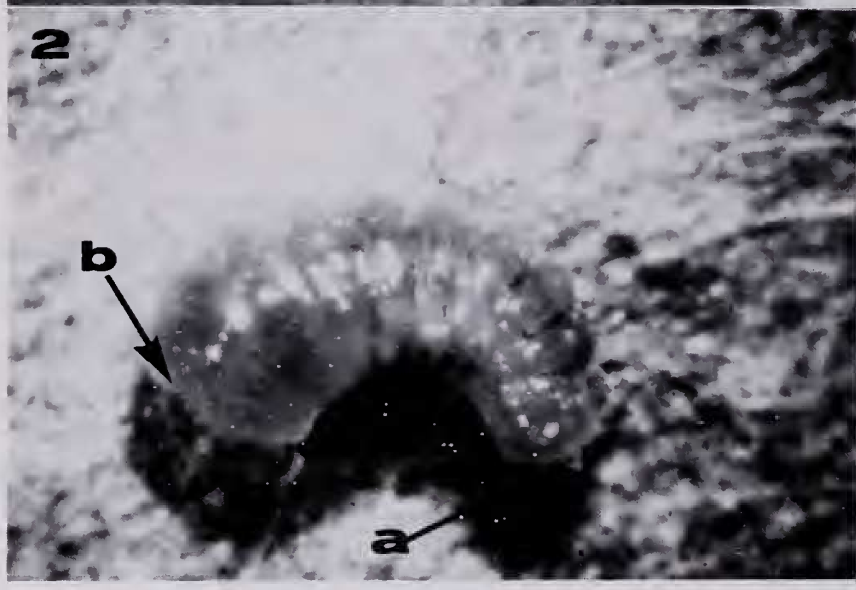


Fig. 1. *Dendrocerus* female (a) backs into the pea aphid mummy (b), drills a hole and oviposits on the *Aphidius* larva developing inside. (20×)

Fig. 2. An 8-day old, 4th instar *Dendrocerus* larva showing the posterior conical process (a) and some of the spine-like projections (b). (55×)

site is usually on the side or rear of the mummy. The female backs into the mummy and anchors herself to the leaf substrate with her prothoracic and sometimes mesothoracic legs (Fig. 1). This is in sharp contrast to *Asaphes* which drills the hole while standing on top of the mummy. The metathoracic legs are hooked into the mummy wall, cornicles, or appendages of the dead aphid. She extends her ovipositor and with a jackhammer-like action drills a hole in the mummy with a twisting and pushing motion. Her antennae are held motionless, being extended forward and usually resting on the leaf. The egg is laid immediately on the primary larva within the mummy. Unlike *Asaphes* (Keller and Sullivan 1976), there is no feeding-tube construction or host-feeding even by a female ovipositing for the 1st time. Two or more eggs are frequently deposited, and although only one larva will normally develop to an adult—occasionally two dwarf-size adult wasps will emerge from the same mummy. The entire ovipositional process may take 3–20 minutes depending on the hardness of the part of the mummy being drilled. In general, however, the time spent searching, antennating, drilling and ovipositing was much shorter in the case of *Dendrocerus* females than with *Asaphes*. The former, therefore, seems to be a much more aggressive and efficient hyperparasitoid.

In maintaining the *Dendrocerus* colony under laboratory conditions, the sex ratio of the adult progeny was 62.8% females (3,622) to 37.2% males (2,149).

The results of the laboratory experiments on tertiary parasitism are given in *Table 2*, where the emergence of *Dendrocerus* No. 2 was greatest on Day 4 (22.4%). On this day, *Dendrocerus* No. 1 is a 2nd instar larva. Later tertiary parasitism was reduced by almost one-half on Days 5 and 6 to 12.1%, at which time the larva of *Dendrocerus* No. 1 is in the 3rd instar. It should be pointed out, however, that even at these stages of *Dendrocerus* No. 1 development, it may be that it is the primary *Aphidius* larva which is really being attacked. This is because the 2nd and even the 3rd instars of the 1st *Dendrocerus* are still relatively small, and true tertiary parasitism may not be occurring.

The remaining test-days revealed an even more dramatic change. Beginning with Day 7, when *Dendrocerus* No. 1 is in the 4th instar, the emergence of *Dendrocerus* No. 2 decreased even further to an average of 6.0%, and remained fairly constant over Days 7–15. The totals for the entire series of 12 test days (4–15) showed that of 1,383 adult wasps which emerged during these experiments, only 8.0% were *Dendrocerus* No. 2, which indicates a relatively low percentage of successful tertiary parasitism.

One explanation for these results might be the defensive behavior of the larva beginning with Day 7. Observations of dissected mummies revealed that on this test-day, the larva of *Dendrocerus* No. 1 is in the 4th instar, and was not merely larger, but unlike earlier instars, each segment had a

Table 2. Adult emergence and non-emergence (mortality) of *Dendrocerus* No. 1 vs. *Dendrocerus* No. 2, arranged according to attack on mummies during the 12 Test-days (4–15).[a]

Test-day	*Dendrocerus* No. 1		*Dendrocerus* No. 2		Total	Mortality[b]		Total
	No.	%	No.	%	emerged	No.	%	mummies
4	59	77.6	17	22.4	76	26	25.4	102
5	102	87.9	14	12.1	116	7	5.7	123
6	102	87.9	14	12.1	116	21	16.0	137
7	110	94.0	7	6.0	117	18	13.3	135
8	104	94.5	6	5.5	110	14	11.3	124
9	107	93.9	7	6.1	114	15	11.6	129
10	106	95.5	5	4.5	111	24	17.8	135
11	110	92.4	9	7.6	119	28	19.0	147
12	111	94.1	7	5.9	118	23	16.3	141
13	109	93.2	8	6.8	117	25	17.6	142
14	129	95.6	6	4.4	135	21	13.5	156
15	124	92.5	10	7.5	134	22	14.1	156
Totals	1273	92.0	110	8.0	1383	244	15.0	1627

[a] Days 0–1–2–3 not included explained in text.

[b] Refers to those mummies from which no *Dendrocerus* emerged.

number of rows of small, lateral and dorsal spine-like projections. In addition, there was a distinctive conical process at the posterior end (Fig. 2) which was also visible in the prepupa. This conical process could even be extended beyond the length visible in the photograph. In parallel experiments on tertiary parasitism presently being conducted with *Asaphes*, this other hyperparasitoid does not possess such a conical process. Perhaps this is the reason why tertiary parasitism with this species is quite high.

Related to this morphological difference between *Dendrocerus* and *Asaphes*, there is also a noticeable difference in behavior. The 4th instar larva and prepupa of *Dendrocerus* No. 1 are easily disturbed when touched either by the ovipositor of a 2nd *Dendrocerus* or even by a mechanical probe. Such prodding resulted in the larva or prepupa twitching violently and seeming to use the conical process as a lever to help it move about in the mummy and away from the stimulus. This is not the case with *Asaphes*.

Hence, it is suggested that, unlike *Asaphes*, the *Dendrocerus* larva has evolved a defensive behavior which prevents or at least drastically reduces tertiary parasitism, at least by its own species. The net result of this ability of the 4th instar larva and prepupa to react to the probing of an ovipositor and even to move about in the mummy by means of the conical process serves as a 94% successful deterrent against hyperparasitization by a 2nd *Dendrocerus* during these stages of development beginning with Day 7.

The pupal stage also seems relatively immune to tertiary parasitism. However, as Sullivan (1972) pointed out when *Asaphes californicus* was used in experiments of tertiary parasitism on another hyperparasitoid, *Alloxysta victrix*, as the pupa becomes sclerotized, tertiary parasitism is greatly reduced. This is probably also the case with *Dendrocerus*.

As shown in Table 2, the mortality or non-emergence of any adult from mummies used in these experiments was rather high, the overall average being 15.0%. This compares favorably, however, with the 18.0% reported by Sullivan in the experiments mentioned above. After being held for several months, these *Dendrocerus* mummies were dissected. All that was visible was a dark-brown, unidentifiable mass on the floor of the mummy. This indicated that diapause was not involved, but rather death of the larvae occurred, due to competition or ovipositional probing.

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Footnotes

[1] Hymenoptera: Ceraphronidae.

[2] This research was supported in part by a National Science Foundation Grant GU-3554 to the second author. Manuscript is a portion of a dissertation submitted by the first author in partial fulfillment of the requirements for the Ph.D. degree in the Department of Biological Sciences, Fordham University, Bronx, New York 10458.

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